In silico protein structure prediction of the γ-secretase: a key enzyme to Alzheimer's disease

A Project Thesis Submitted in Partial Fulfillment of The

Requirment for The Degree in

Bachelor of Technology

In Biotechnology

Submitted by:-

Ritiritao Brahma

109BT0667

Under the Supervision of:-

Dr. Nandini Sarkar

Assistant Professor

Department of Biotechnology and Medical Engineering

National Institute of Technology, Rourkela

Odisha-769008

National Institute of Technology, Rourkela

Odisha-769008

CERTIFICATE

This is to certify that the project report entitle "**In silico structure prediction of γ-secretase: a key enzyme in Alzheimer's disease**" submitted by **RITIRITAO BRAHMA** (109BT0667) in the partial fulfillment of the requirement for the degree of the B.Tech in Biotechnology Engineering in Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela is an authentic work carried out by him under my supervision. To the best of my knowledge the matter embodied in the report has not been submitted to any other Institute/University for any degree.

Date: - 6th May, 2013

Place: - Rourkela

Dr. Nandini Sarkar (Supervisor)

Assistant Professor

Department of Biotechnology and Medical Engineering

National Institute of Technology, Rourkela

Odisha-769008

ACKNOWLEDGEMENTS

I would like to take this opportunity to extent my hearty gratitude to my guide and advisor Dr. Nandini Sarkar, Assistant Professor; Department of Biotechnology and Medical Engineering; National Institute of Technology-Rourkela,Odisha-769008, whose constant guidance and encouragement made the completion of my B.Tech thesis possible.

I also thank National Institute of Technology Rourkela, for permitting me to utilize the facilities in its laboratories to carry out my experiment.

I would also like to thank Arun Evr, M.Tech for giving me constant support with all the necessary things involve in this whole project.

Submitted by

Ritiritao Brahma

Roll No.-109BT0667

Department of Biotechnology & Medical Engineering

National Institute of Technology-Rourkela, Odisha-769008

Table of Contents

Certificate ……..i

Acknowledgment…….....ii

List of figures…….2

List of tables……...……....3

Abstract……..4

1. Introduction……....5

1.1. Cause of the Disease…….6

1.2. Harmful effects……....7

1.3. Therapeutic Approach……..…...8

1.4. Objective…….……9

2. Literature Review……..…….10

3. Materials and Methods…….……11

3.1. Amino Acid Sequence Search…….……..11

3.2. BLAST…….……14

3.3. Secondary Structure prediction…….……..….14

3.4. I-TASER Prediction……..…15

3.5. Structure Validation……..19

3.6. Energy Minimization……...23

3.7. Docking……26

4. Results and Discussion……..31

5. Conclusion……35

6. Reference……...36

List of Figures

Figure.1: Breakdown of APP by γ-secretase enzyme……..…..6

Figure.2: Representation of various sub units of γ-secretase……...9

Figure.3: I-TASSER structure prediction of PEN-2 sub Unit……..16

Figure.4: I-TASSER structure prediction of Nicastrin sub unit……..17

Figure.5: I-TASSER structure prediction of Aph1 sub unit…….18

Figure.6: I-TASSER structure prediction of Presenilin sub unit…….19

Figure.7: Selected Models viewed using Chimera……...23

Figure.8: Energy Minimization of selected models…….25

Figure.9: Energy Minimization of selected models…….26

Figure.10: Docking Result of Aph1-Presenilin……27

Figure.11: Resultant Structure of the Docking of Aph-1 and Presenilin…….28

Figure.12: Docking result of Aph-1 and Presenilin with the Nicasrin sub unit……...28

Figure.13: Resultant structure of the docking……..32

Figure.14: Docking of Aph-1 + Presenilin + Nicastrin with Pen-2…….29

Figure.15: Resultant structure of the docking of all the sub units……30

Figure.16: Docking Result comparison between two ways applied in docking…….…..31

Figure.17: Structure comparison between the two different docking ways…….31

Figure.18: Ramachandran Plot of the tertiary structure complex predicted…….32

List of Tables

Table.1: Secondary Structure prediction by Chou & Fasman……..……..15

Table.2: Coaparison of different models of Pen-2 Sub unit……..…….....16

Table.3: Comaprison of different models of Nicastrin sub unit…….……17

Table.4: Comparison of different models of Aph-1 sub unt…….……..18

Table.5: Ramachandran Plot analysis…….21

Table.6: SwissPDB astructure assessment…….22

Table.7: Ramachandran plot analysis of the tertiary structure……...…34

ABSTRACT

Alzheimer's disease is one of the most common forms of neurodegenerative disease in which the extracellular deposition of fibrils and tangles of the aggregation of amorphous amyloid β-peptide are found in some part of the brain, mainly in the region responsible for learning, memory and emotional behavior. Neurons that use glutamate and acetylcholine as neurotransmitter are mainly affected. The disease is caused due to the deposition of Aβ amyloid extracellular. The protein AAP is break down into Aβ amyloid fragments by γ-secretase and α-secretase enzyme that in later stage forms aggregate. Although α- secretase has its chemical structure but γ-secretase which has four subs unit structure has yet not been found. Here we will try to predict the structure of these subunits though *Ab Initio* structure prediction method. And using the subunit structure we will try to predict complex structure.

Keywords: Alzheimer, neurodegenerative, amyloid, amyloid precursor protein, gamma secretase, ab initio

1. INTRODUCTION

Alzheimer's disease (AD) is one of the most common forms of dementia. The disease mainly affects the neuron cells in the brain. Accumulation of plaques and tangles are found in the AD patience's brain [1, 2]. The plaques are extracellular deposition of fibrils and amorphous aggregation of the amyloidβ-peptide in high amount. Neurofibrillary tangles are intracellular fibrillar aggregation of the microtubules associated tau proteins that are required for the neurons growth and maintenance [2]. The plaques and tangles are mainly present in that region of the brain which are responsible for learning, memory and emotional behaviors such as hippocampus, entorhinal cortex, amygdala and basal forebrain [1]. Brain regions with plaques and tangles exhibit reduced number of synapse and the neurons which use glutamate and acetylcholine as neurotransmitter are found to be mostly affected.

Persons with AD show mild forgetfulness and trouble remembering recent events at the beginning and as the disease progresses the person starts losing any sense, like unable to remember direction, names, and unable to recognize their own once at late stage [http://en.wikipedia.org/wiki/Alzheimer's_disease].

The most dangerous about this disease is that, there is still no drug that can cure AD. Although there are some drugs that can relatively decrease the progress the development of the disease as per U.S. Food and Drug Administration (FDA).

1.1. Cause of the Disease

The real cause of this disease is yet not found out but it has been seen that the one of the main cause of this disease is the accumulation of amyloid β-peptide protein in some part of the brain. Amyloid β-peptide fragments aggregate leading to form fibril like structure which has an irreversible cross β structure [34]. Amyloid β-peptides are form from the breakdown of APP (amyloid precursor protein) by γ-secretase, α-secretase and β-secretase enzyme. But involvement α-secretase remains unclear [35]. APP is a transmembrane protein which has many isoforms ranging in size from 695 to 700 amino acids. The most abundant form of insoform APP695 found in brain in produced by neuron cell [1].

Cleavage of APP by α-secretase releases sAPPα from the cell surface and leaves an 83-amino-acid carboxy-terminal APP fragment (C83). Amyloidogenic processing of APP involves sequential cleavages by β-secretase and γ-secretase at the N and C terminal of Aβ respectively. The 99-amino-acid C-terminal fragment of APP cleaved by β-secretase.

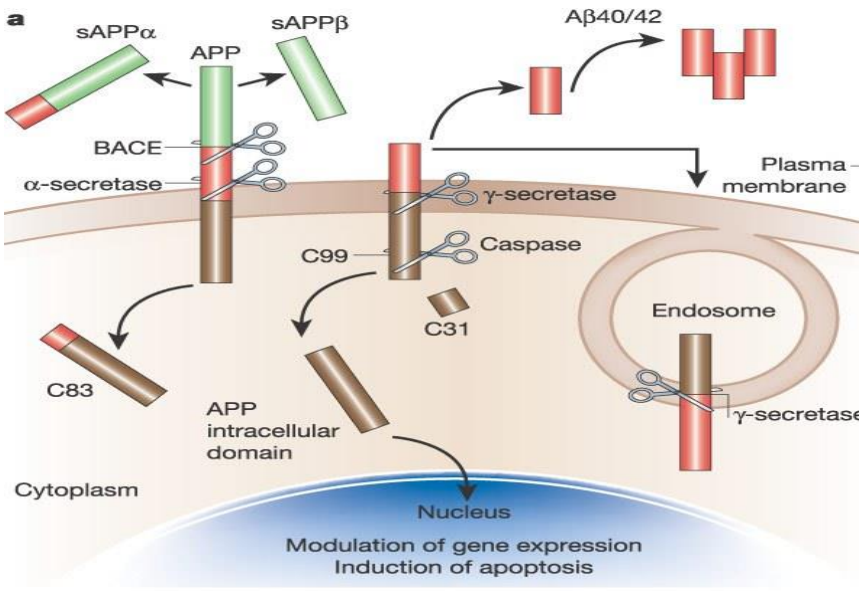


Figure.1. - Breakdown of APP by β-secretase and γ-secretase enzyme and formation of Aβ40/42, key fragment to the amyloid aggregation.

Cleavage can be internalized and further processed by γ-secretase to produce Aβ40/42 in extracellular. Cleavage of C99 by γ-secretase liberates an APP intracellular domain that can translocate to the nucleus where it may regulate gene expression, including the induction of apoptotic genes. Cleavage of APP/C99 by caspases produces a neurotoxic peptide (C31)67. Aβ40/42 fragment forms aggregate and in later stage an irreversible fibril like structure which has a cross β-sheet structure. This irreversible fibril like structure accumulates in some part of the brain mainly in synaptic junctions where the presence of APP is more.

AD tough is not inherited from parents but through molecular genetics it is found that the individual that produces more apoliprotein E4 has higher risk of getting AD. The mechanism whereby E4 promotes AD is not established, but there is evidence that E4 enhances Aβ aggregation and reduces Aβ clearance. In addition, data suggest that E4 might increase the risk of AD by enhancing amyloidogenic processing of APP, increasing oxidative stress and impairing neuronal plasticity [7].

Other factor that may enhance the susceptibility of AD to a person is the mutation in APP, PS1 and PS2 genes. Mutation in these genes increases the production of long 42 amino acid form of Aβ (Aβ42) part in APP.

1.2. Harmful Effects

APP is required for the growth and maintenance of the neuron cells. Perturbed processing of APP resulting in increased production of Aβ at synapses may be an early event in AD. APP is axonally transported and Aβ therefore likely accumulates at synapses in high amounts in AD. Aβ can have multiple adverse effects on the functions and integrity of both pre- and postsynaptic terminals including inducing oxidative stress, impairing calcium homeostasis and perturbing the functions of mitochondria and the ER. The increased Aβ deposition that occurs in AD most probably contributes to the demise of neurons because Aβ can be directly

toxic to neurons and also greatly increases their vulnerability to oxidative and metabolic stress, and toxicity.

Another effect of accumulation of Aβ peptide extracellular is the induced production of highly oxidative free-radicals. The neurotoxic action of Aβ involves generation of reactive oxygen species and disruption of cellular calcium homeostasis. Interactions of Aβ oligomers and Fe^{+2} or Cu^{+} generate hydrogen peroxide. When Aβ aggregation occurs at the cell membrane, membrane-associated oxidative stress results in lipid peroxidation and the consequent generation of 4-hydroxynonenal (4HNE), a neurotoxic aldehyde that covalently modifies proteins on cysteine, lysine and histidine residues. Oxidative modifications of tau protein by 4HNE and other reactive oxygen species can promote its aggregation and may thereby induce the formation of neurofibrillary tangles. Aβ can also cause mitochondrial oxidative stress and deregulations of Calcium homeostasis, resulting in impairment of the electron transport chain, increased production of superoxide anion radical and decreased production of ATP.

Fe^{+2} or Cu^{+} generates the hydroxyl radical (OH), a highly reactive oxyradical and potent inducer of membrane-associated oxidative stress that contributes to the dysfunction of the ER.

1.3. Therapeutic approach

The γ-secretase, which cleaves APP within a transmembrane region, involves four different proteins, presenilin, nicastrin, Aph-1 and Pen-2. The active site of γ-secretase requires the aspartyl protease activity of PS1 conferred by aspartate residues in adjacent transmembrane domains of the C- and N-terminal cleavage fragments of PS1 (red star). Nicastrin, Pen-2 and Aph-1 are each critical components of γ-secretase and each may modify enzyme activity in

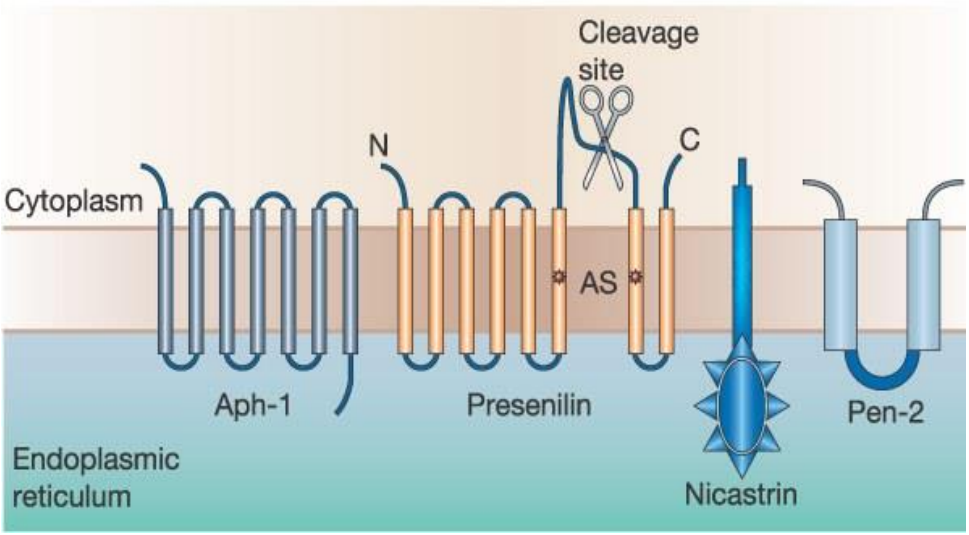


Figure.2: - Representation of the four sib units of γsecretase enzyme, namely Aph-1, Presenilin, Nicastrin and Pen-2

specific ways and in response to physiological stimuli. APP is only one of several proteins that are cleaved by γ-secretase.

The best way to stop this AD is either we inhibit the γ-secretase and β-secretase enzyme or we inhibit the aggregation of amyloid β-peptide or reverse the fibril structure. But to inhibit the β-secretase and γ-secretase enzyme, we must know the structure of the enzyme. The structure of β-secretase is known but the structure of γ-secretase and its four sub unit is still not known, although structure of presenilin is in CASP12 list.

1.4. Objective

- The objective of this project is 'to predict the structure of sub units of γ-secretase enzyme and using this structure to predict the structure of the complex γ-secretae enzyme'.

2. LITERATURE REVIEW

The main objective is to predict the structure of the γ-secretase protein using the best known method available. Homology modeling method is best for those sequences which have template similarity more than 80% [Chung SY et al, 1996, 126-141]. *Ab initio* modeling [8–10], method is best in terms of the accuracy but success is limited to small proteins with < 120 amino acids [3, 11]. And from the literature review it is found that during the CASP experiment the I-TASSER method have shown significant advantages of composite approaches in protein structure prediction [11, 13], which combine various techniques such as threading, *ab initio* modeling and atomic-level structure refinement approaches[13, 17].

I-TASSER (iterative threading assembly refinement) [10], an automated protein structure prediction method has been listed as the best method for the automated protein structure prediction in the last two CASP experiments [13, 17]. I-TASSER assisted by the sequence profile and the predicted secondary structure, the query sequence is then threaded through a representative PDB structure library using LOMETS, a locally installed meta-threading server combining seven state-of-the-art threading programs. The model selected is done Monte Claro Stimulation to select the best model.

Detailed descriptions of the I-TASSER methodology for protein structure and function prediction have been in following steps-

Threading > structure assembly > model selection and refinement > structure based functional annotation.

In here we have done differently with PDB-BLAST, and in structure selection we have used Ramachandran plot [K.Gopalakrishna et al, 2007, 14,699-671] and Z-score and Docking for the minimized model structures. The secondary structure generated was compared with the annotated structure information given for each of the four sub units.

3. MATERIALS & METHODS

1.1.Amino Acid Sequence Search:

The amino acid sequence of sub units; presenilin, nicastrin, pen2 and aph1 are searched in the NCBI database. NCBI (http://www.ncbi.nlm.nih.gov/) database is part on United States National Library of Medicines, a branch of National Institute of Health. Sequence of all the sub units are searched using Entrez search engine.

1. Amino acid Sequence of PEN-2 sub unit in FASTA format: >gi|28144920|ref|NP_758844.1| gamma-secretase subunit PEN-2 [Homo sapiens]

MNLERVSNEEKLNLCRKYYLGGFAFLPFLWLVNIFWFFREAFLVPAYTE QSQIKGYVWRSAVGFLFWVIVLTSWITIFQIYRPRWGALGDYLSFTIPLG TP

2. Amino acid Sequence of APH-1 subunit in FASTA format : >gi|344313184|ref|NP_001230700.1| gamma-secretase subunit APH-1A isoform 3 [Homo sapiens]

MGAAVFFGCTFVAFGPAFALFLITVAGDPLRVIILVAGKADEGLASLSED GRSPISIRQMAYVSGLSFGIISGVFSVINILADALGPGVVGIHGDSPYYFLTS AFLTAAIILLHTFWGVVFFDACERRRYWALGLVVGSHLLTSGLTFLNPW YEASLLPIYAVTVSMGLWAFITAGGSLRSIQRSLLCKD

3. Amino acid sequence of Nicastrin subunit in FASTA format:

>gi|9992878|gb|AAG11412.1|AF240468_1 nicastrin [Homo sapiens]

MATAGGGSGADPGSRGLLRLLSFCVLLAGLCRGNSVERKIYIPLNKTAPC VRLLNATHQIGCQSSISGDTGVIHVVEKEEDLQWVLTDGPNPPYMVLLES KHFTRDLMEKLKGRTSRIAGLAVSLTKPSPASGFSPSVQCPNDGFGVYSN

SYGPEFAHCREIQWNSLGNGLAYEDFSFPIFLLEDENETKVIKQCYQDHN
LSQNGSAPTFPLCAMQLFSHMHAVISTATCMRRSSIQSTFSINPEIVCDPLS
DYNVWSMLKPINTTGTLKPDDRVVVAATRLDSRSFFWNVAPGAESAVAS
FVTQLAAAEALQKAPDVTTLPRNVMFVFFQGETFDYIGSSRMVYDMEKG
KFPVQLENVDSFVELGQVALRTSLELWMHTDPVSQKNESVRNQVEDLLA
TLEKSGAGVPAVILRRPNQSQPLPPSSLQRFLRARNISGVVLADHSGAFHN
KYYQSIYDTAENINVSYPEWLSPEEDLNFVTDTAKALADVATVLGRALYE
LAGGTNFSDTVQADPQTVTRLLYGFLIKANNSWFQSILRQDLRSYLGDGP
LQHYIAVSSPTNTTYVVQYALANLTGTVVNLTREQCQDPSKVPSENKDLY
EYSWVQGPLHSNETDRLPRCVRSTARLARALSPAFELSQWSSTEYSTWTE
SRWKDIRARIFLIASKELELITLTVGFGILIFSLIVTYCINAKADVLFIAPRE
PGAVSY

4. Amino Acid sequence of Presenilin in FASTA format:

>gi|119601503|gb|EAW81097.1| presenilin 1 (Alzheimer disease 3), isoform CRA_b [Homo sapiens]

MTELPAPLSYFQNAQMSEDNHLSNTVRSQNDNRERQEHNDRRSLGHPEP
LSNGRPQGNSRQVVEQDEEEDEELTLKYGAKHVIMLFVPVTLCMVVVV
ATIKSVSFYTRKDGQLIYTPFTEDTETVGQRALHSILNAAIMISVIVVMTIL
LVVLYKYRCYKVIHAWLIISSLLLLFFFSFIYLGEVFKTYNVAVDYITVAL
LIWNFGVVGMISIHWKGPLRLQQAYLIMISALMALVFIKYLPEWTAWLI
LAVISVYDLVAVLCPKGPLRMLVETAQERNETLFPALIYSSTMVWLVNM
AEGDPEAQRRVSKNSKYNAESTERESQDTVAENDDGGFSEEWEAQRDSH
LGPHRSTPESRAAVQELSSSILAGEDPEERGVKLGLGDFIFYSVLVGKASA

TASGDWNTTIACFVAILIGLCLTLLLLAIFKKALPALPISITFGLVFYFATD

YLVQPFMDQLAFHQFYI

ncbi.nlm.nih.gov > protein database > name of the protein [Homo sapiens]

1.2. BLAST:

Protein-protein BLAST is done for each of the four sub units. But the similarity among the sub units of the similarity is found to be less than 80%. E-value in BLAST search measures the probability that the search result in non-random.

ncbi.nlm.nih.gov > protein database > name of the protein [Homo sapiens] > select the FASTA sequence or Accession number > select PDB protein database > run protein-protein BLAST

1.3. Secondary Structure Prediction:

Secondary structure is predicted using Chou & Fasman online server. This method works on the principle of probability parameters determined from relative frequencies of each amino acid's appearance in each type of secondary structure. The algorithm followed by Chou & Fasman in that it assigns certain probability value to residues weather it will be helix, coil or stand and calculate on the basis of propensity of the structure among certain number of risidues.

Sub Unit	Secondary Structure	Total Residue	Percentage of the total Residue
Presenilin (467 amino acids)	α- helix(H)	365	78.2
	β- sheet(E)	50	10.7
	β-turn(T)	311	66.6
Nicastrin (709 amino acids)	α-helix(H)	459	64.9
	β-sheet(E)	97	13.7
	β-turn(T)	454	64.0
PEN-2 (101 amino acids)	α-helix(H)	74	73.3
	β-sheet(E)	10	9.9
	β-turn(T)	74	73.3
Aph-1 (190 amino acids)	α-helix(H)	148	77.9
	β-sheet(E)	16	8.4
	β-turn(T)	119	62.4

Table.1: - Secondary dtructure prediction of all sub units by Chou & Fasman method

1.4. I-TASSER Prediction Method

I-TASSER (Iterative Threading Assembly Refinement) is a unified platform for automated protein structure and functional prediction based on the sequence to structure and from structure to functional paradigm [Amboish Roy et al, 2005]. The threading is done with the help of a eight state of art threading software called LOMET [Sitao Wu et al, 2007], that includes FUGUE, PROSPECT2, SPARKS2, SAM-To, HHSERACH, PPA-I, PPA-II and

PAINT threading method. All the structures generated by these threading methods are assembled and being analyzed to get the best ten model structures for the sub units.

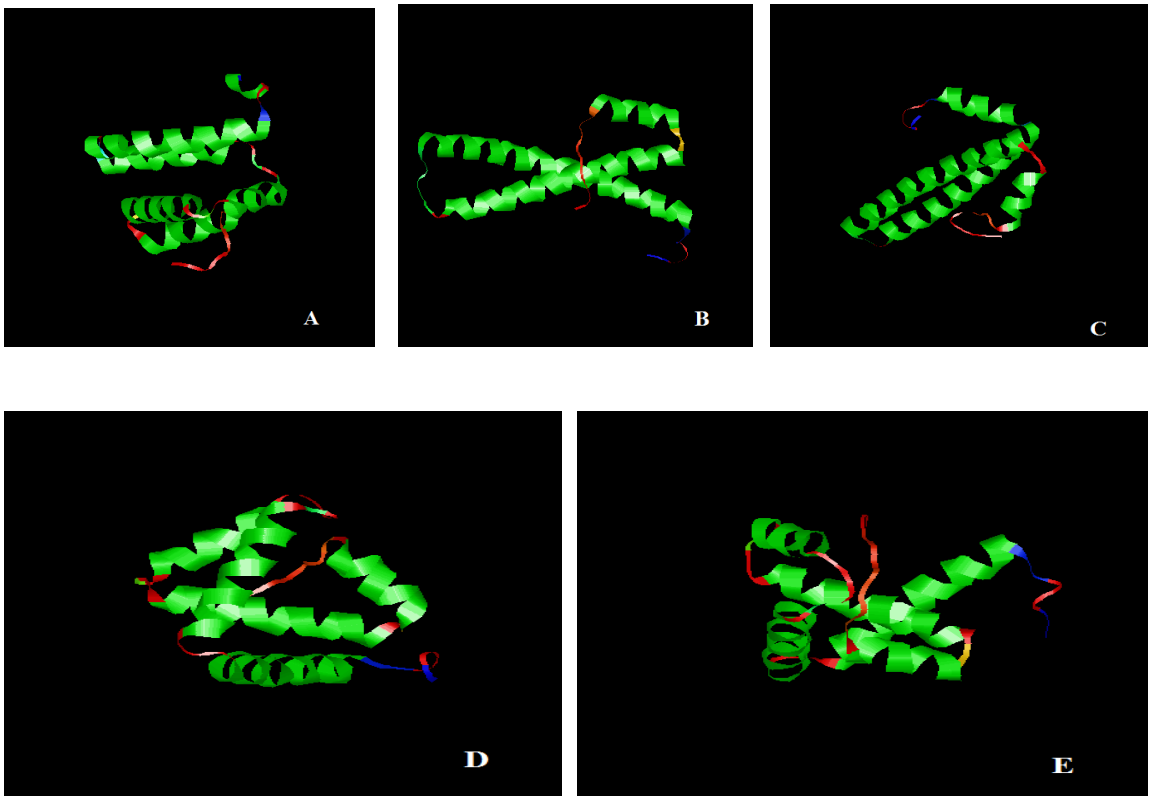


Figure.3:-I-TASSER prediction models for Pen-2 sub unit.

Pen-2 (Number of groups-101, number of atoms-859 & number of bonds-892)	**Number of Helix (H)**	**Number of Turns (T)**	**Number of Sheet (E)**
Model1	6	9	0
Model2	3	2	0
Model3	4	5	0
Model4	5	11	0
Model5	5	8	0

Table.2: - Comparison of different models of Pen-2 sub unit generated by I-TASSER.

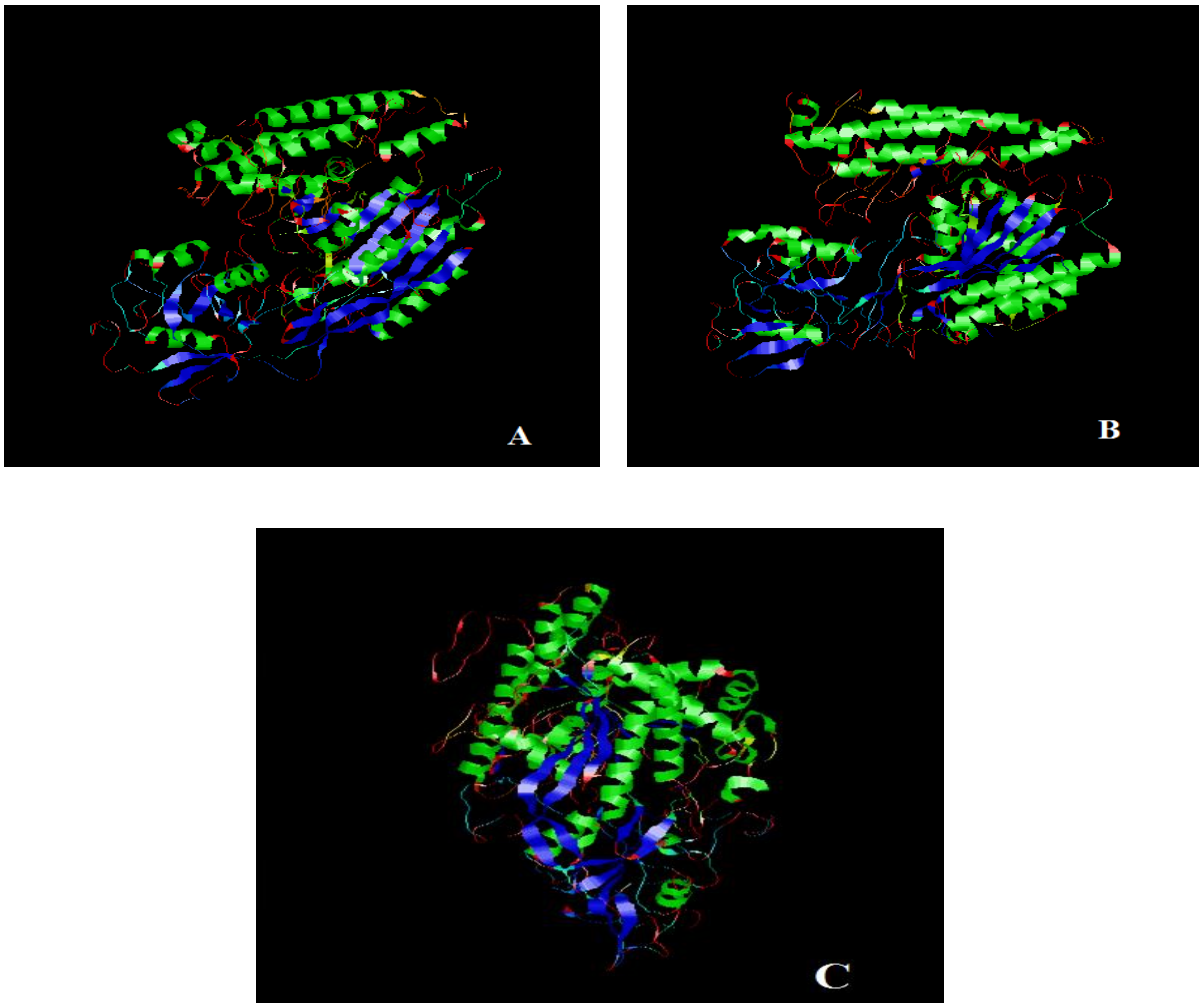


Figure.4: - I-TASSER predicted models for Nicastrin sub unit.

Nicastrin (Number on groups-709, number of atoms-5523, number of -5654)	**Number of Helix(H)**	**Number of Turn (T)**	**Number of Sheet (E)**
Model1	22	78	20
Model2	25	83	21
Model3	22	75	21

Table.3: - Comparison of different models of Nicastrin sub unit generated by I-TASSER.

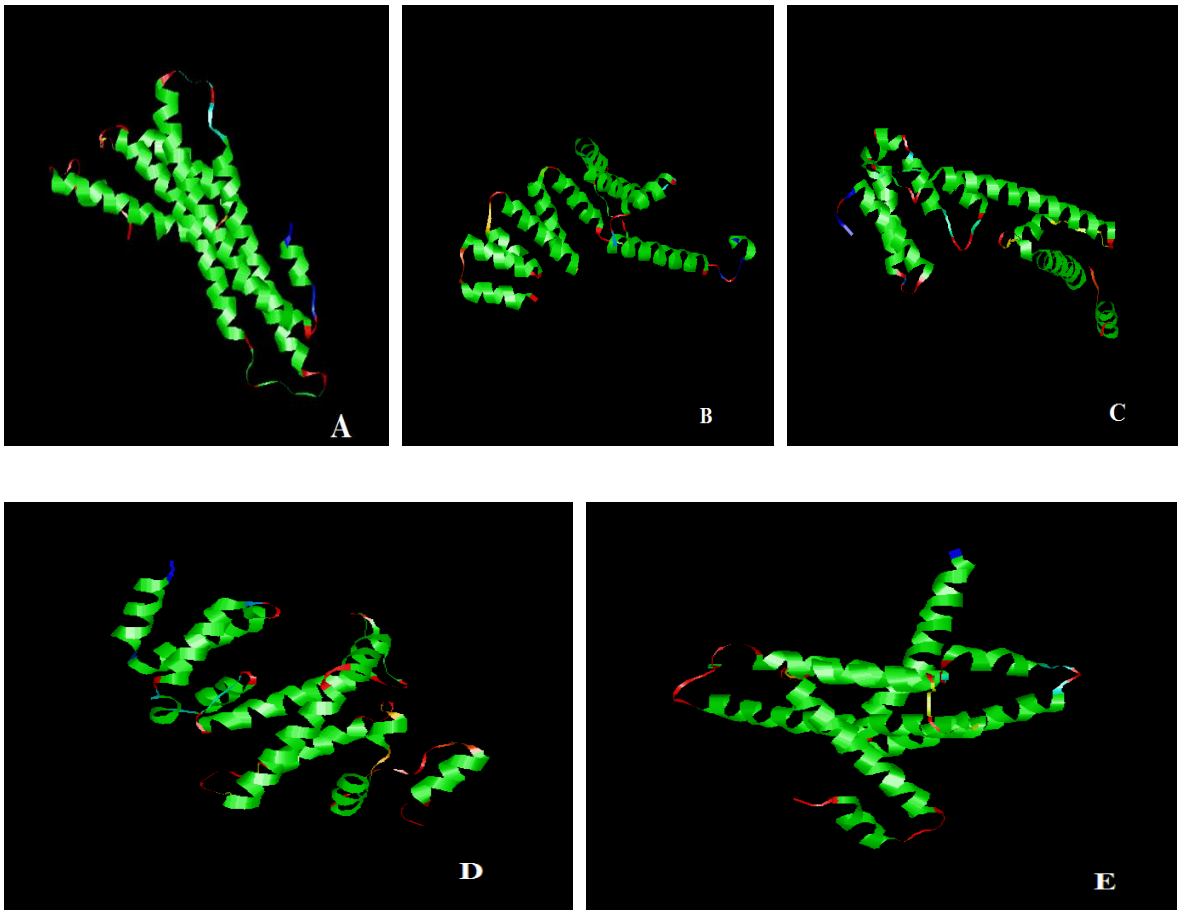


Figure.5: - Different models generated byI-TASSER for Aph-1 sub unit.

Aph1(Number of groups-190, number of atoms-1433 & number of bonds-1471)	**Number of Helix(H)**	**Number of Turn(T)**	**Number of Sheet(E)**
Model1	8	13	0
Model2	12	12	0
Model3	12	13	0
Model4	12	13	0
Model5	8	10	0

Table.4. - Comparison of different models of Aph-1 sub unit generated by I-TASSER.

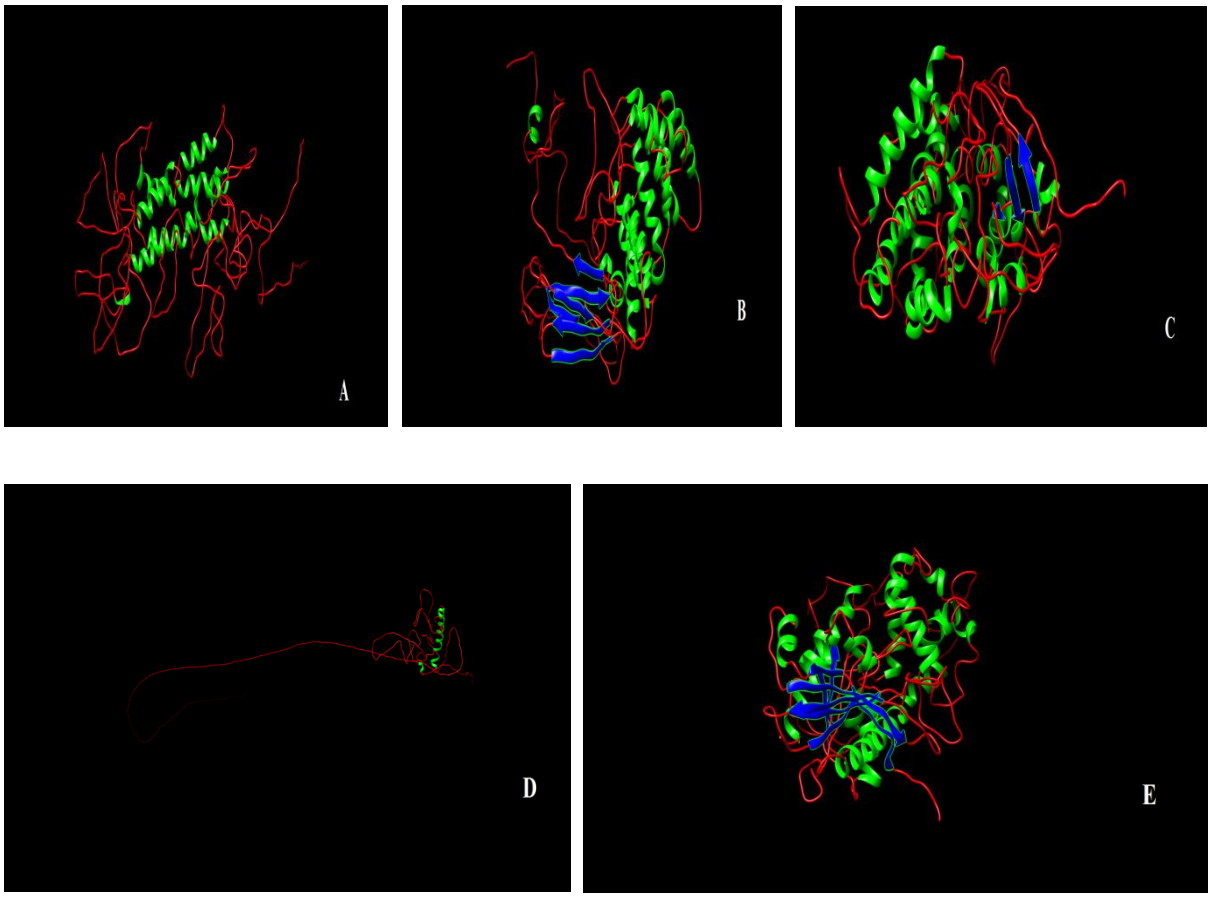


Fig.6: - Sequence of Presenilin sub unit is in CASP12 (Critical Assessment of Protein Structure Prediction 2012), therefore the structure is predicted by BhageeratH, not by I-TASSER.

The structure of each sub unit generated is compared with the annotated structure given in the ChEMBL Uniprot/ SwissProt. Although the annotated structure shows only potential of the secondary structure and thus not indicate the exact structure of the sequence. Comparing the structure predicted using I-TASSER method, the structures are having similarity with annotated structure information.

1.5. STRUCTURE VALIDATION

1.5.1. Ramachandran Plot

A Ramachandran plot is a way to visualize backbone dihedral angle ψ (psi) against φ (pi) of amino acid residues in protein structure. Ψ angle is the angle between the bonds from the α-carbon to the carbonyl group (at the C-terminus) of the amino acid. The φ angle is angle between the bonds from the nitrogen (at the N-terminus) to the α-carbon of the amino acid residue. We can vary ψ from –180° to 180° and we can vary φ from –180° to 180° (that is 360° of rotation for each). But all the angles in a protein cannot be rotated due many constrains, like steric resistance, partial double bond characters etc.

Understanding the steric limitations in individual amino acid residues reveals how these limitations result in the observed types of secondary structures found in nature.

The complex folding of proteins is controlled, in a large part, by the limitations on the ψ and φ angles available to each amino acid residue. Two main amino acids that contribute in the Ramachandran plot are Glycine and Proline. Glycine does not have steric hindrance to other 19 amino acids. Proline residues are conformationally restricted due to the ring being part of the backbone. In general practice, glycine and proline residue data is not plotted in Ramachandran plots.

Though the plot of the models we can find out which models has highest number of residue in the allowed region and favored region. Based on the number of residue in the allowed region and favored region the models are being selected for the Energy minimization.

Sub unit	Models	Favored region	Allowed region	Outlier region
Aph-1	Model1	172	11	5
	Model2	174	8	6
	Model3	173	12	3
	Model4	167	17	6
	Model5	176	8	4
Pen-2	Model1	96	2	1
	Model2	94	5	0
	Model3	94	2	3
	Model4	88	8	3
	Model5	88	6	5
Nicastrin	Model1	581	74	52
	Model2	568	82	57
	Model3	583	71	53
Presenilin	Molde1	273	166	16
	Model2	288	158	9
	Model3	326	119	10
	Model4	258	184	13
	Model5	345	106	14

Table.5: Ramachandran Analysis of different models

1.5.2. Swiss PDB Structure assessment

SwissPDB structural assessment is based on the **Qmean6** value and the **Z-score.** Qmean6 value is a global scoring function [*Benkert et al. 2008*] is a linear combination of six structural descriptors using statistical potentials: analyze torsion angle potential and distance-dependent interaction potentials. A solvation potential investigates the burial status of the residues. Two additional terms describing the agreement of the predicted (from sequence) and the calculated secondary structure and solvent accessibility of the model.

Z-scores of the Qmean composite score provides relative quality estimates to scores obtained for high-resolution reference structures solved experimentally by X-ray crystallography [Benkert et al. 2011]. The Qmean and Z-score represents a measure of the absolute quality of a model. Models of low quality are expected to have strongly negative Qmean Z-scores.

Sub unit	Assessment Values	Model1	Model2	Model3	Model4	Model5
Aph-1	Qmean value	0.388	0.292	0.090	0.259	0.302
	Z-score	-3.91	-4.90	-6.96	-5.24	-4.79
Pen-2	Qmean value	0.354	0.305	0.273	0.414	0.352
	Z-score	-3.53	-3.97	-4.27	-2.98	-3.97
Presenilin	Qmean value	0.435	0.246	0.239	0.209	0.401
	Z-score	-3.35	-4.21	-4.58	-4.37	-2.90
Nicastrin	Qmean value	0.350	0.262	0.298		
	Z-score	-4.57	-5.54	-5.15		

Table.6: - SwissPDB structural assessment of different models.

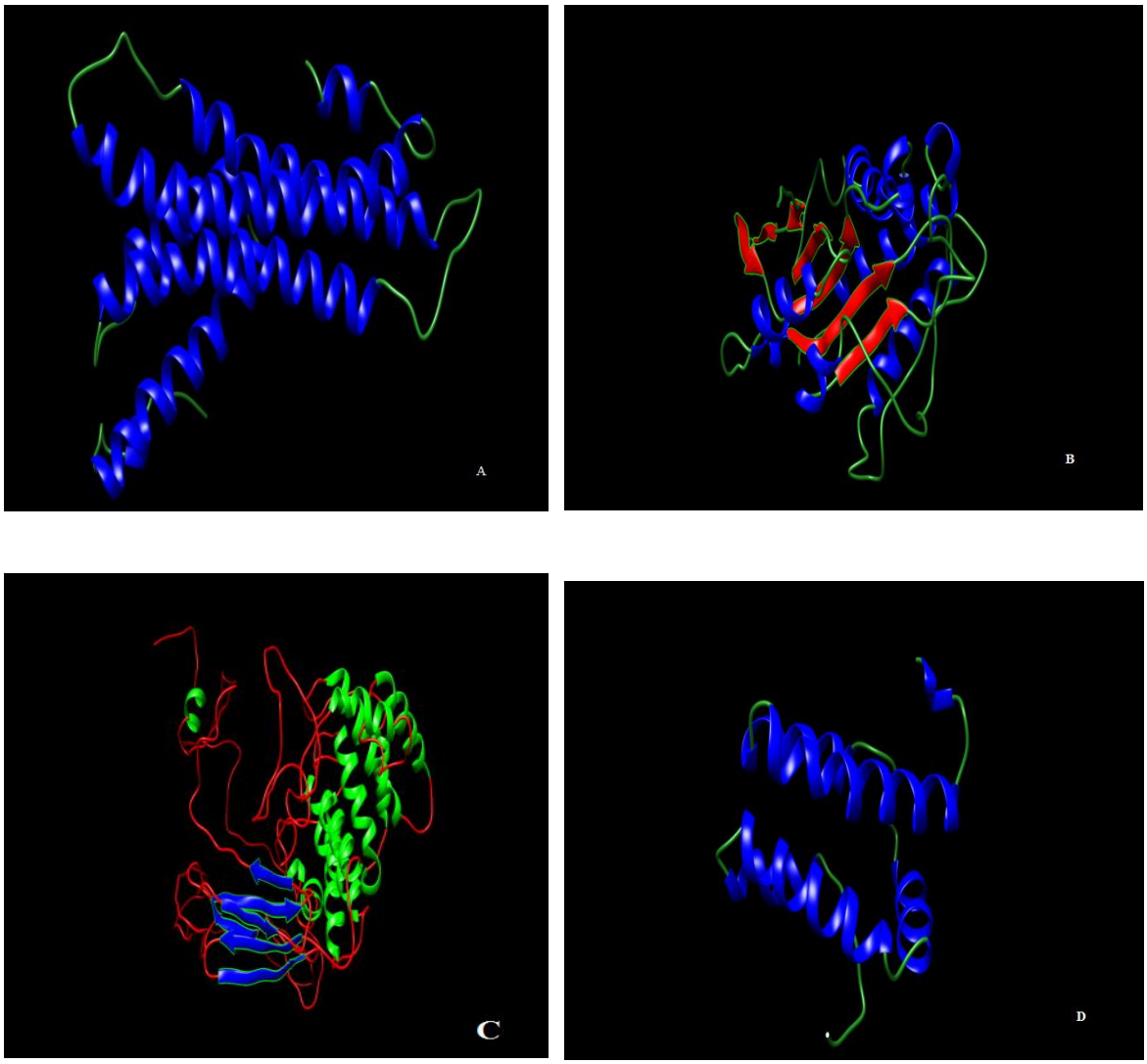


Figure.7: - Selected I-STRUCTURE models viewed with Chimera of four sub units (A) Aph-1, (B).Nicastrin (C). Presenilin and (D). Pen-2 with red, blue and green colors representing strand, helix and coil respectively.

1.6.Energy Minimization

Stable states of molecular systems correspond to global and local minima on their potential energy surface. Starting from a non-equilibrium molecular geometry, energy minimization employs the mathematical procedure of optimization to move atoms so as to reduce the net forces, the gradients of potential energy on the atoms until they become negligible. Like molecular dynamics and Monte-Carlo approaches, periodic boundary conditions have been allowed in energy minimization methods, to make small systems.

There are two methods for minimization of energy of a protein structure model.

1. **Steepest Descent Method**

This method uses the first derivative to determine the direction towards minimum. The energy is calculated at the initial geometry and again calculated in after one of the atoms is moved in a small increment in one of the direction of the coordinate system. The process in continued for each atom until the predetermined minimum threshold energy is reached.

2. **Conjugate Gradient Method**

This technique uses information from previous derivatives to determine the optimum direction for line search. The gradient is calculated at each step which serves as additional procedure for better minimization. Hence each step refines the direction towards the minimum and thus this method follows after the Steepest Descent method.

In here we have used the same methods for minimization of the energy of the selected models using the Chimera Software. In this software first steepest descent method is done and then conjugates gradient method in done.

During the minimization step, the water molecules, ligands which are bind to the protein molecule are being removed and the Hydrogen bond is added to the structure to make it more stable.

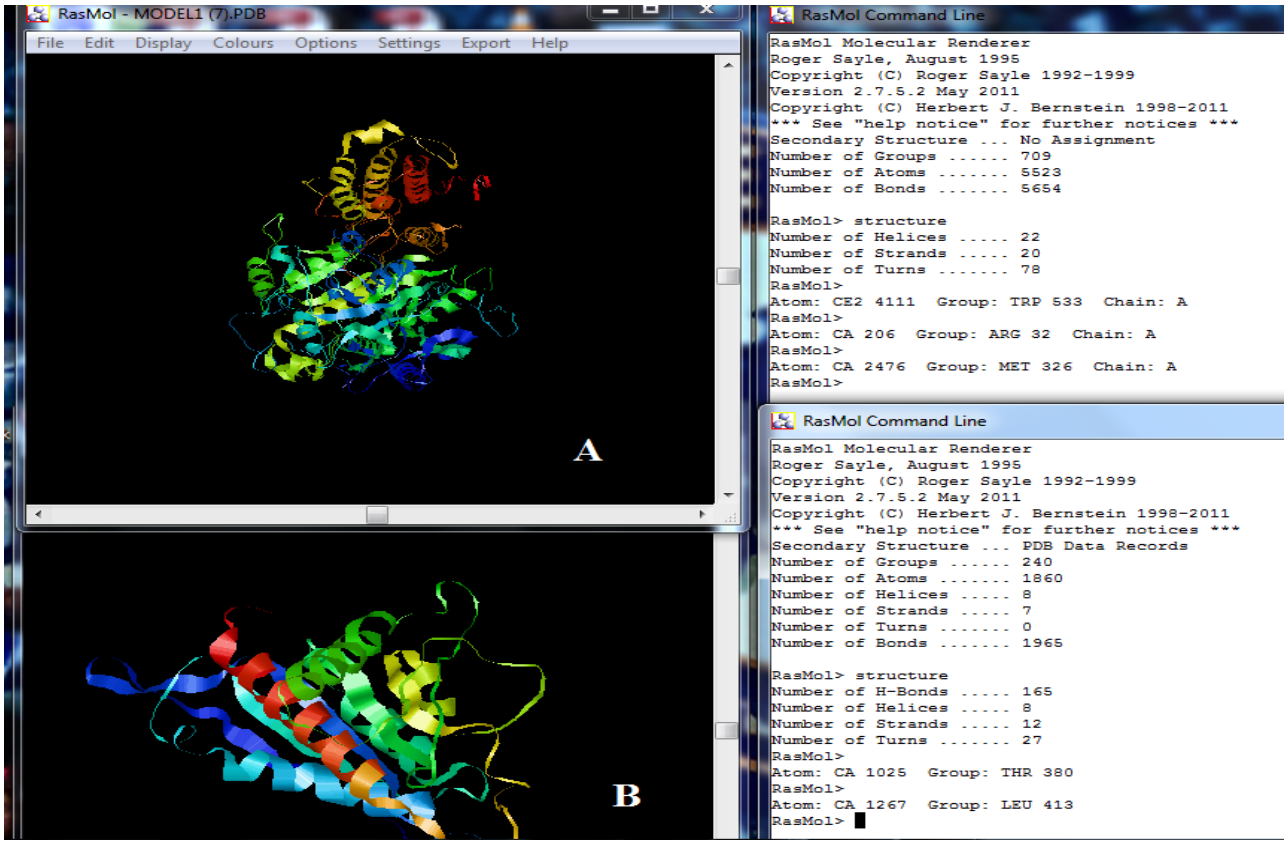
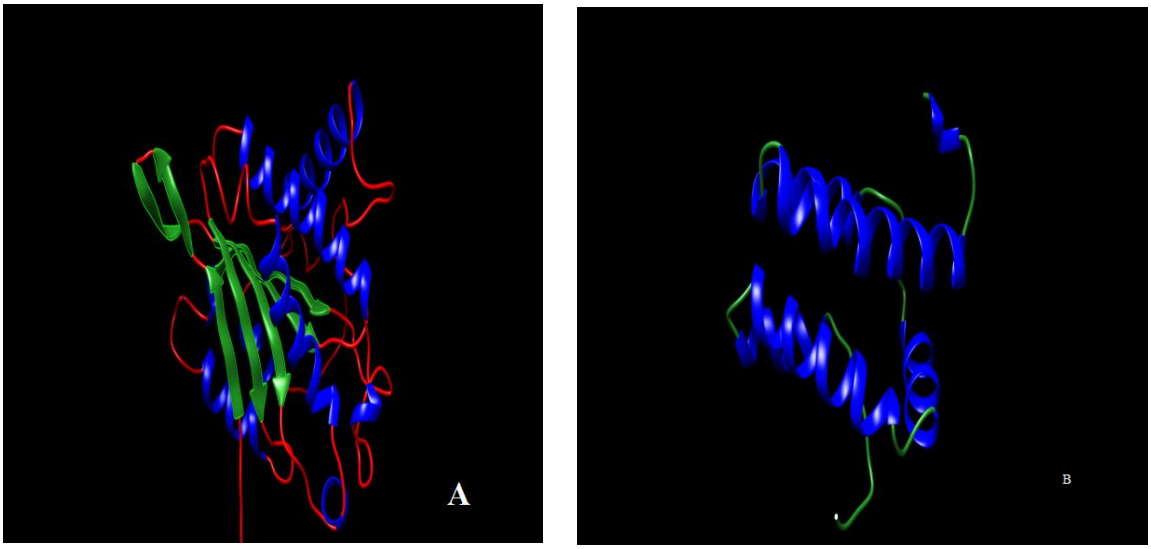


Figure.8: - (A). Example showing comparison between the template model structures (Without energy minimization) and (B).the minimized structure of the same structure.



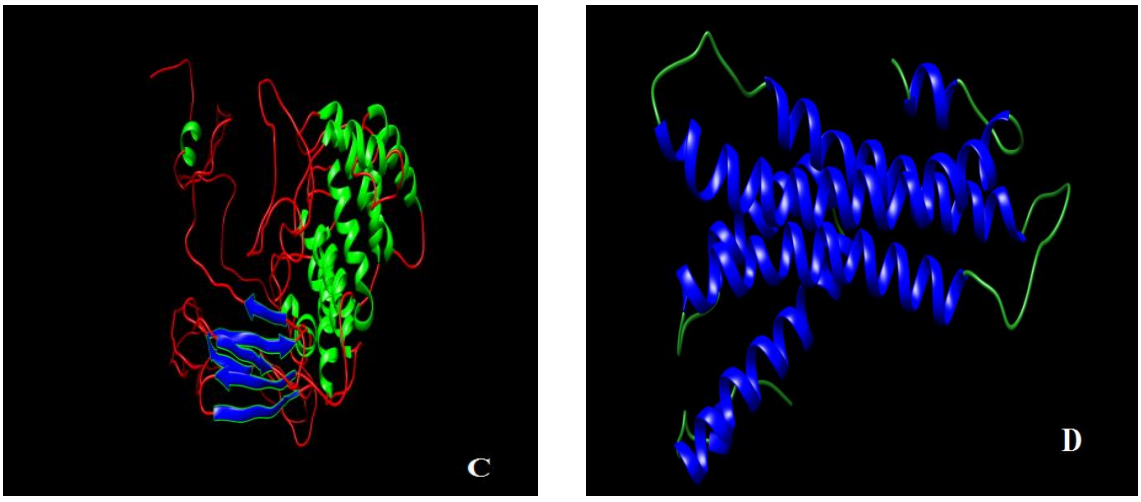


Figure.9: - Minimized structure selected models of the (A).Nicastrin, (B).Pen-2, (C).Presenilin and (D). Aph-1

1.7. DOCKING

Docking is the method to determine the interaction between different molecules. We can predict the active site of the molecules where another molecule can come and bind to it. The orientation of the molecules which is preferred can also be determined [Lengauer T et al, (1996)]. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. The interaction can be of many types like-

1. Protein-protein
2. Ligand-protein
3. Ligand-ligand

1.7.1. Docking Using Hex6.3 software

Hex is an interactive protein docking and molecular superposition program, written by Dave Ritchie. *Hex6.3* understands protein and DNA structures in PDB format, and it can also read small-molecule SDF files.

The docking was done in a sequential manner [Figure.2 represents the sequence of sub unit]. Since the Aph-1 remains towards the surface it has been docked with the Presenilin. Then the resultant structure was docked with the Nicastrin. Than the resultant of the from these three sub units was docked with Pen-2 sub unit.

In second case the sequence of docking was just change from the other side, i.e. Pen-2 with Nicastrin and the resultant structure was docked with Presenilin sub unit. Then the resultant structure of these three sub units was docked with the Aph-1 sub unit.

1st Docking step: - Aph-1 sub unit with Presenilin sub unit

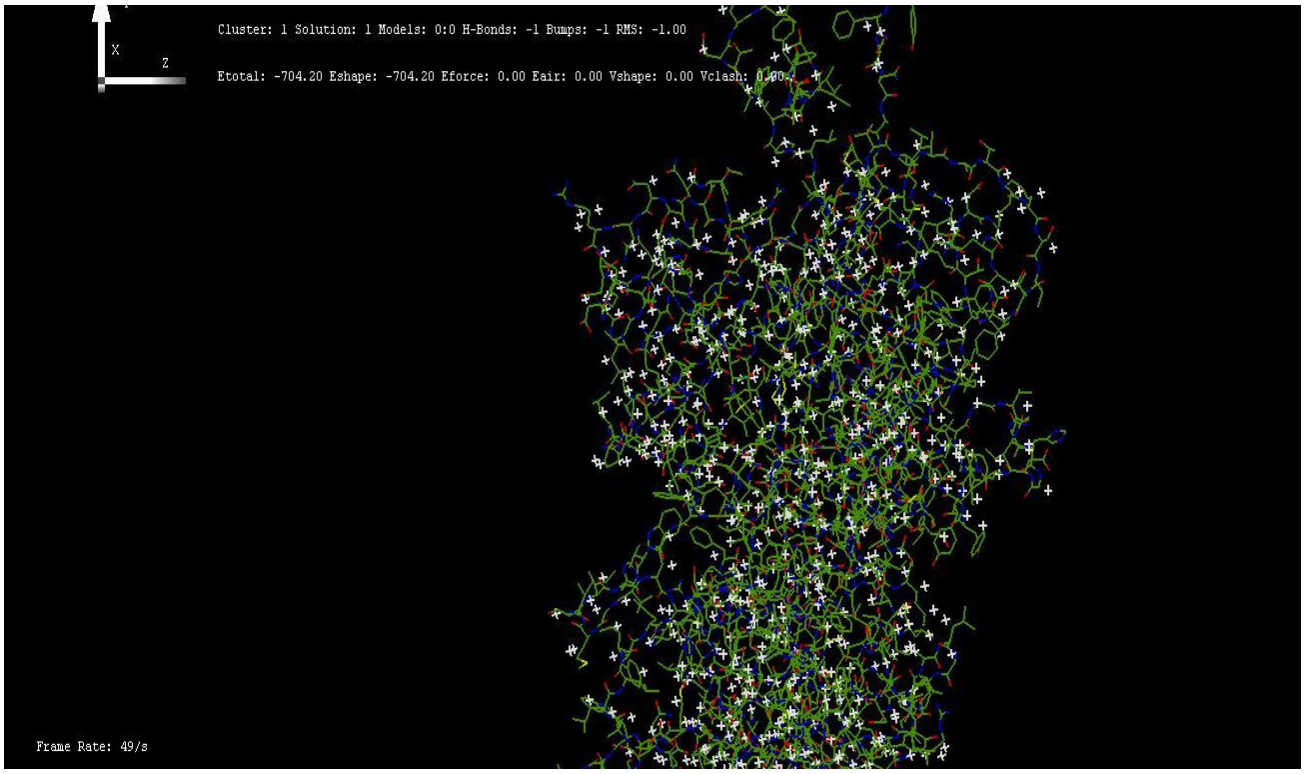


Figure.10: Docking result between Aph-1 and Presenilin sub unit. The energy was obtained as -704.20kJ.

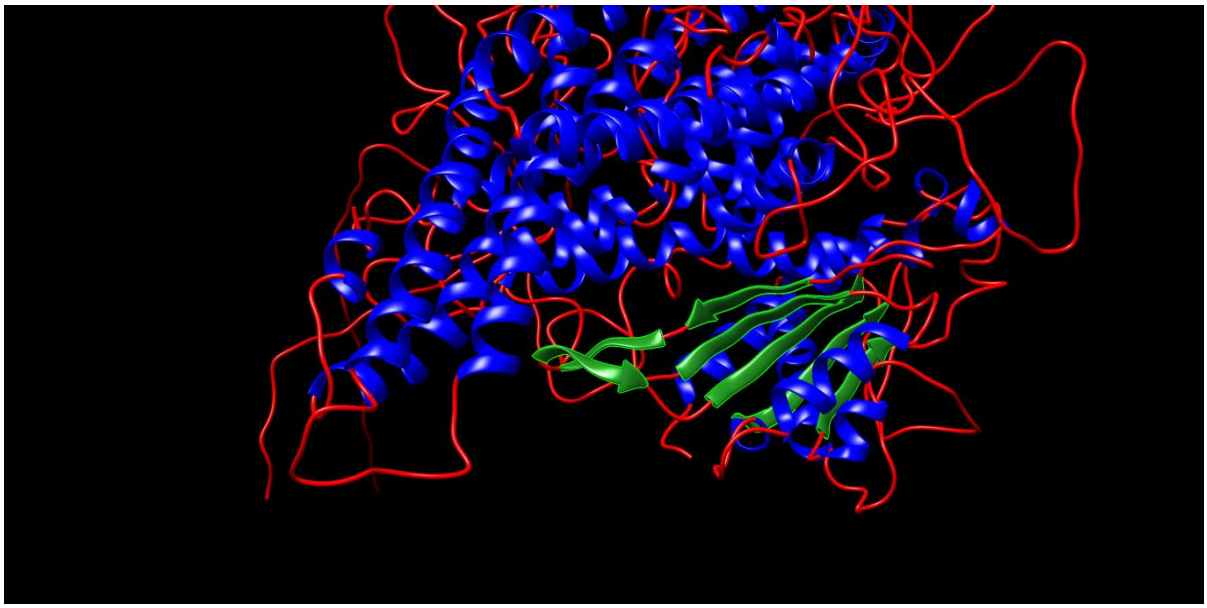


Figure.11:- The resultant structure of the docking between Aph-1 and Presenilin

2nd docking step: The resultant structure from Ahp-1 and Presenilin with Nicastrin sub unit

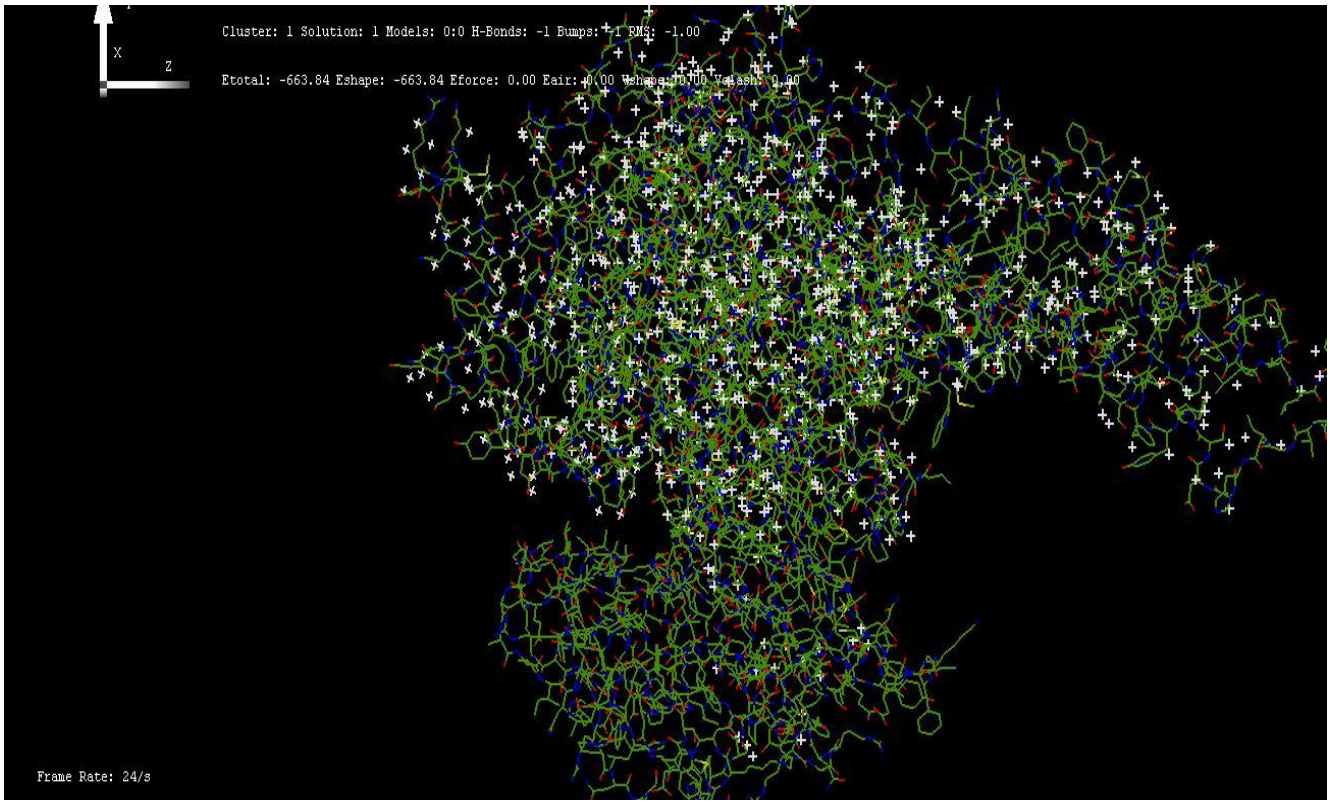


Figure.12: Docking result of Aph-1 and Presenilin with Nicastrin. The energy was found to be -663.84kJ

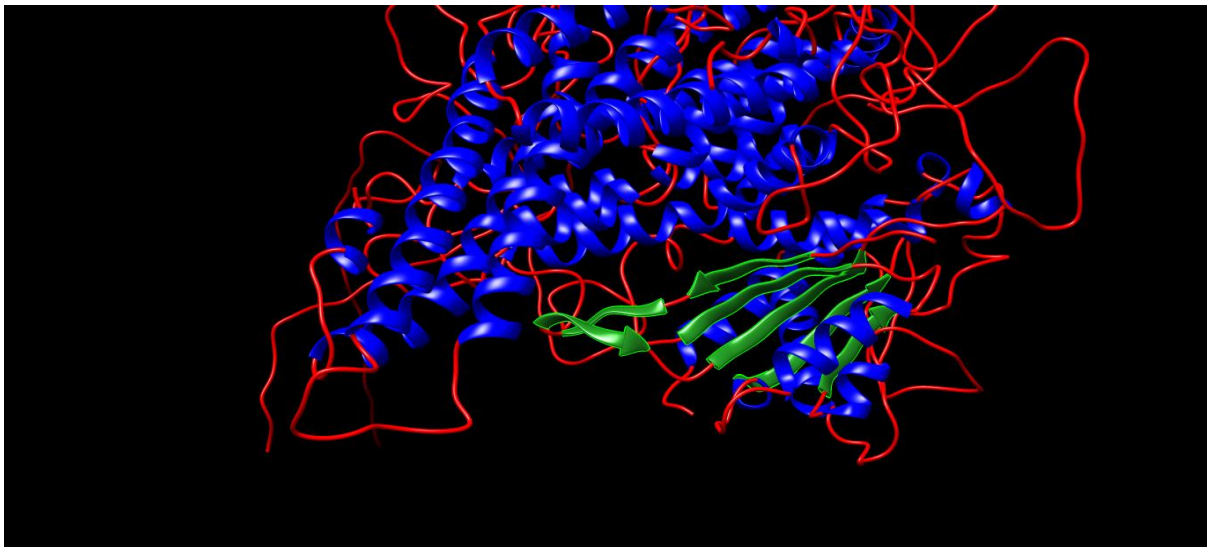


Figure.13: Resultant structure of the docking between Aph-1 and Presenilin with Nicastrin

3rd Docking step: The resultant structure of Aph-1, Presenilin and Nicastrin with Pen-2 sub unit.

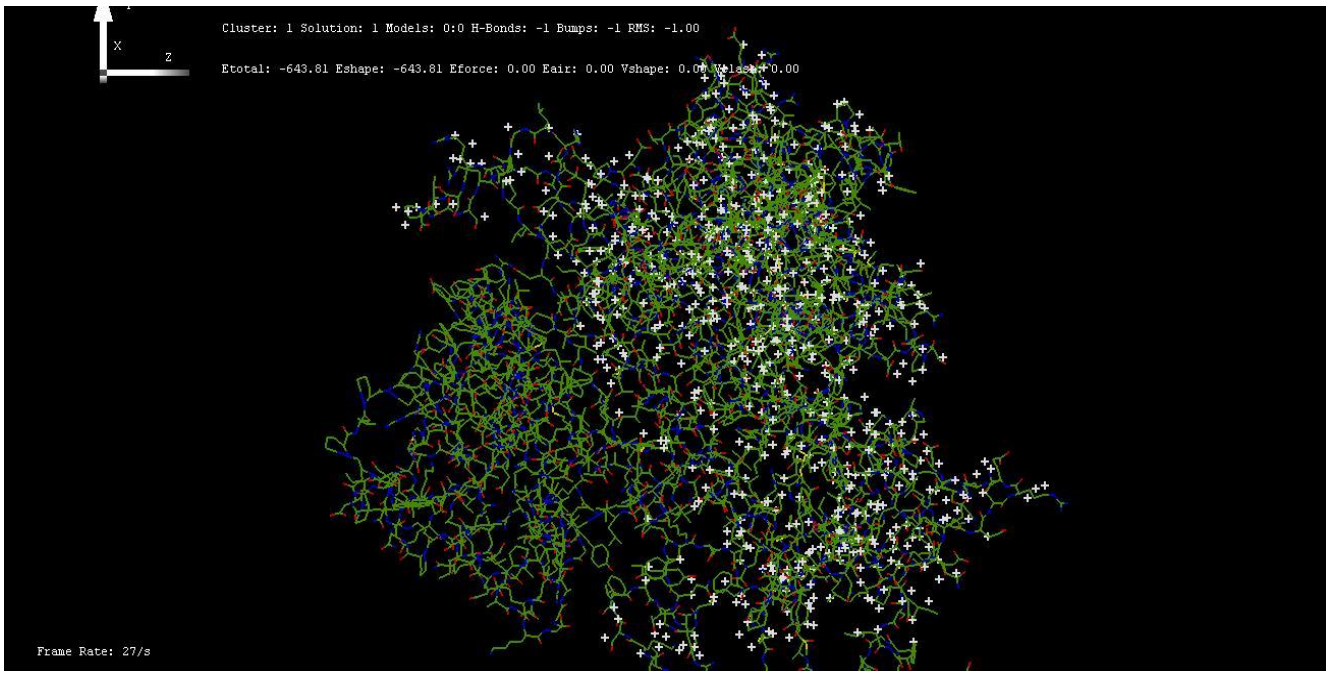


Figure.14: - Docking result of all the four sub units with energy of -643.81 kJ

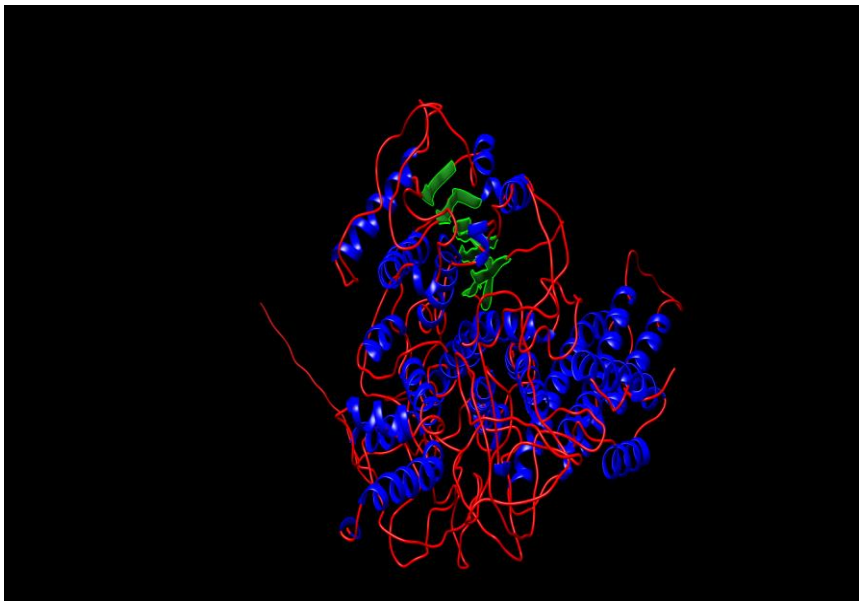


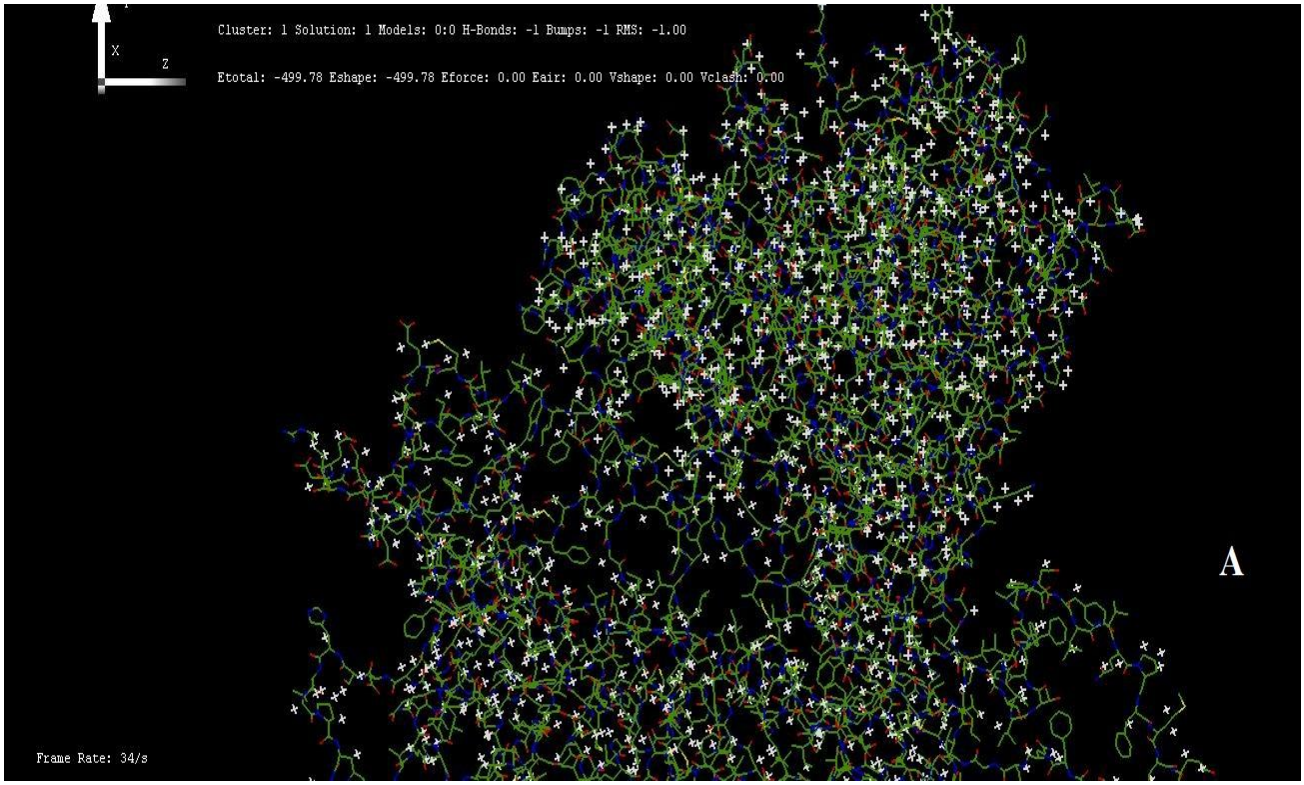
Figure.15: The final docking resultant structure of all the sub units.

4. RESULTS AND DISCUSSION

4.1. RESULT

Using the I-TASSER method we have got secondary structure for three sub units, but for Presenilin, the I-TASSER couldn't generate the secondary structure because the sequence is listed in the CASP12(Critical Assessment of Protein Structure) project. The secondary structures generated are almost similar to that has been annotated in the EMBL-UniprotKB.

The final docking result from two different ways performed is having different energy level. The final docked using (((Aph-1+Presenilin)+Nicastrin)+Pen-2) was found to be -643.81kJ, whereas for the (((Pen-2+Nicastrin)+Presenilin)+Aph-1) resulted into an energy of -499.78kJ.



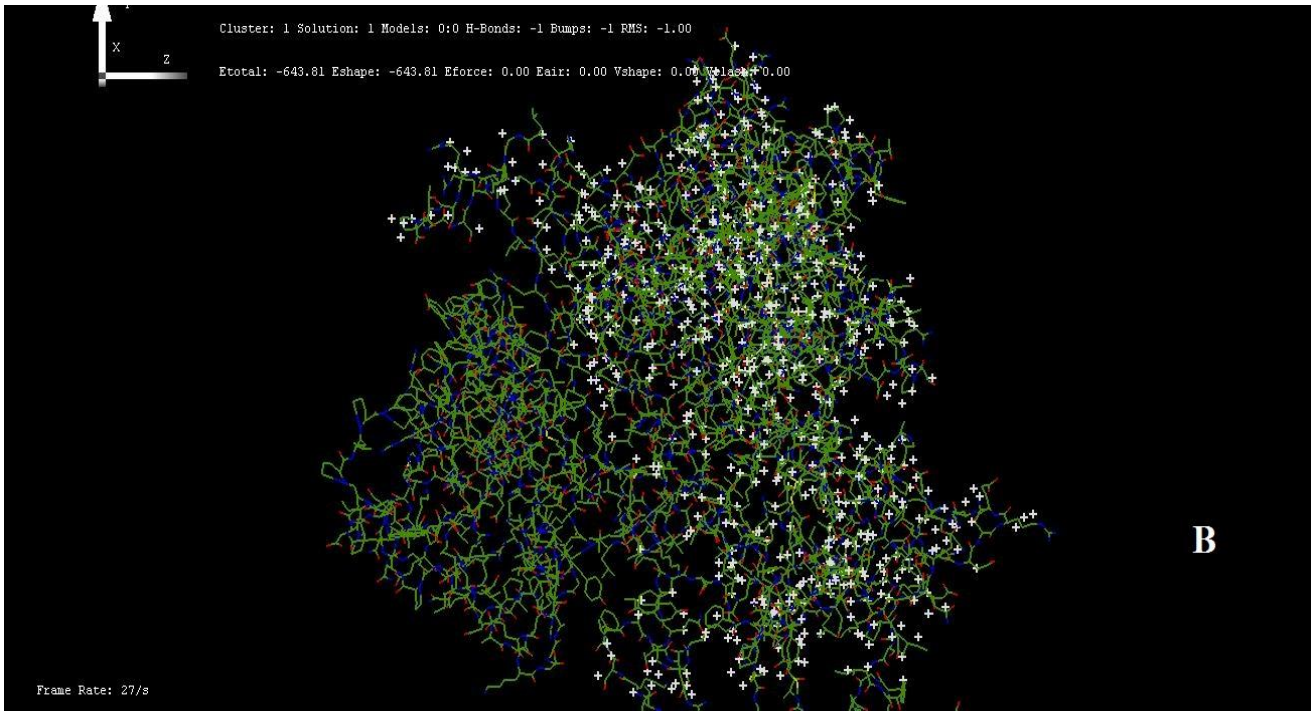


Figure.16: (A). Shows the Docking result from Pen-2 with Nicastrin with Presenilin and then with Aph-1, (B). Shows the docking result from Aph-1 with Presenilin and then with Nicastrin and at last with Pen-2

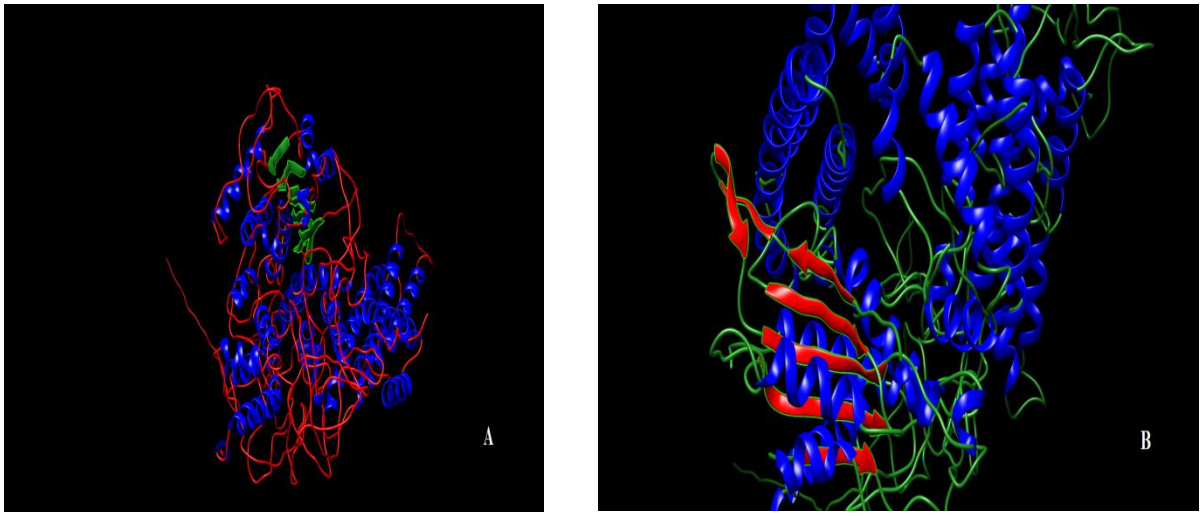


Figure.17: (A). structure generated by Aph-1 with Presenilin and then with Nicastrin and then with Pen-2 (B). Structure generated via Pen-2 with Nicastrin, then with Aph-1

4.2. DISCUSION

The secondary structure prediction was not having similarity more than 80%, therefore we couldn't do the Homology modeling. Using the Modeller software also the Script for the secondary prediction shows no topology for the given sequence. Thus the I-TASSER method was best for such sequences which use the threading method to recognize the fold of particular protein sequence. The secondary structure for Presenilin was not generated by I-TASSER because in CASP12 (Critical Assessment of Protein Structure Prediction 2012), Presenilin has been taken for experimental determination of the structure. The secondary structure for the Presenilin was predicted by BhageeratH online server.

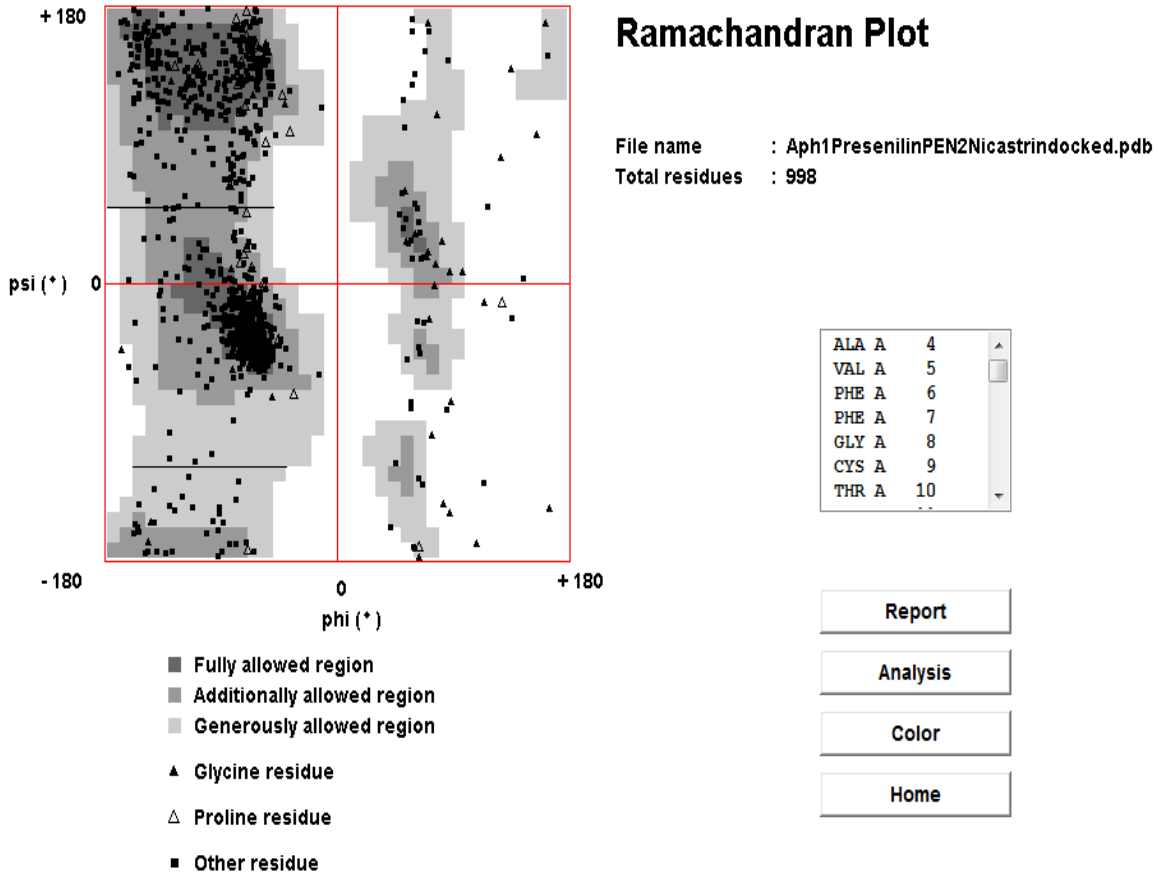


Figure.18: Ramachandran Plot of the tertiary structure generated from all the sub units.

From the Ramachandran Plot analysis of the structure generated was done with the favored region, allowed region and the outlier region which shows the structure generated is good.

Docked result of all the sub units (998 residues)	**Number of Residue**	**Percentage of the total**
Favored Region	**664**	**67.07**
Allowed Region	**301**	**30.16**
Outlier Region	**25**	**2.53**

Table.7: Ramachadran Plot analysis of the tertiary structure generated using all the sub units of the γ-seretase enzyme

The secondary structure generated using the I-TASSER method and that of the annotated information about the secondary structure in the ChEMBL Uniprot/ SwissProt are similar in nature.

The structure annotation is the potential structure for that particular sequence. And the structure predicted is having similarity but in case if Nicastrin the similarity is less indicating that there is error in the method or the annotation.

5. CONCLUSION

The tertiary structure of the γ-secretase enzyme is being predicted with most number of residues in the favored region in Ramachandran Plot. The residue in favored region and the allowed regions are fine with 67.07% and 30.16% of the total residue respectively. This indicates good interaction the of sub unit structures to form the complex structure of γ-secretase enzyme.

The secondary structure predicted has similarity compared to the annotated structure information in SwissProt/ChEMBL UniProt.

But the structure we have generated is not the perfect structure of the γ-secretae enzyme. The dynamics of each atoms present in the structure needs to be studied and the other stimulations are also to be considered. Orientation of each bonds in the structure needed Flexible Docking which takes three to four days in a single docking.

6. REFERENES

1. Dickson,D.W. Neuropathological diagnosis of Alzheimer's disease: a perspective from longitudinal clinicopathological studies. Neurobiol. Aging 18, S21–S26 (1997).
2. Braak, H. & Braak, E. Evolution of neuronal changes in the course of Alzheimer's disease. J. Neural Transm. Suppl. 53, 127–140 (1998).
3. Selkoe, D. J. & Schenk, D. Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. Annu. Rev. Pharmacol. Toxicol. 43, 545–584 (2003).
4. Hardy, J. Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci. 20, 154–159 (1997).
5. Sherrington, R. et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375, 754–760 (1995).
6. Levy-Lahad, E. et al. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 269, 973–977 (1995).
7. Roses, A. D. A model for susceptibility polymorphisms for complex diseases: apolipoprotein E and Alzheimer disease. Neurogenetics 1, 3–11 (1997).
8. Tanzi, R. E.& Bertram, L.New frontiers in Alzheimer's disease genetics.Neuron 32, 181–184 (2001).
9. Mayeux, R. Epidemiology of neurodegeneration. Annu. Rev. Neurosci. 26, 81–104 (2003).
10. Mattson, M. P. Gene-diet interactions in brain aging and neurodegenerative disorders. Ann. Intern. Med. 139, 441–444 (2003).
11. Young, D. et al. Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. Nature Med. 5, 448–453 (1999).

12. Lee, J., Duan,W. & Mattson, M. P. Evidence that brain-derived neurotrophic factor is required for basal neurogenesis andmediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. J. Neurochem. 82, 1367–1375 (2002).
13. Cotman, C.W. & Berchtold, N. C. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci. 25, 295–301 (2002).
14. Bush, A. I., Masters, C. L. & Tanzi, R. E. Copper, beta-amyloid, and Alzheimer's disease: tapping a sensitive connection. Proc. Natl Acad. Sci. USA 100, 11193–11194 (2003).
15. Haass, C. et al. The Swedish mutation causes early-onset Alzheimer's disease by beta-secretase cleavage within the secretory pathway. Nature Med. 1, 1291–1296 (1995).
16. Scheuner, D. et al. Secreted amyloid beta-protein similar to that in senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 APP mutations linked to familial Alzheimer's disease. Nature Med. 2, 864–870 (1996).
17. Mattson, M. P. Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives. Physiol. Rev. 77, 1081–1132 (1997).
18. Morgan, D. et al. A beta peptide vaccination prevents memory loss in an animal model of
19. Alzheimer's disease. Nature 408, 982–985 (2000).
20. Butterfield, D. A. et al. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide. Trends Mol. Med. 7, 548–554 (2001).
21. Smith, M. A. et al. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc. Natl Acad. Sci. USA 94, 9866–9868 (1997).

22. Blass, J. P. Brain metabolism and brain disease: is metabolic deficiency the proximate cause of Alzheimer dementia? J. Neurosci. Res. 66, 851–856 (2001).
23. Dodart, J. C. et al. Early regional cerebral glucose hypometabolism in transgenic mice
24. overexpressing the V717F beta-amyloid precursor protein. Neurosci. Lett. 277, 49–52 (1999).
25. Buchner, M., Huber, R., Sturchler-Pierrat, C., Staufenbiel, M. & Riepe, M. W. Impaired hypoxictolerance and altered protein binding of NADH in presymptomatic APP23 transgenic mice. Neuroscience 114, 285–289 (2002).
26. Watson, G. S. & Craft, S. The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. CNS Drugs 17, 27–45 (2003).
27. Gabuzda, D., Busciglio, J., Chen, L. B., Matsudaira, P. & Yankner, B. A. Inhibition of energy metabolism alters the processing of amyloid precursor protein and induces a potentially amyloidogenic derivative. J. Biol. Chem. 269, 13623–13628 (1994).
28. Saito, K. et al.Widespread activation of calcium-activated neutral proteinase (calpain) in the brain in Alzheimer disease: a potential molecular basis for neuronal degeneration. Proc. Natl Acad. Sci. The UniProt, C. The Universal Protein Resource (UniProt) 2009. *Nucleic Acids Res.* **37**, D169–D174 (2008).
29. Berman, H.M. *et al.* The Protein Data Bank. *Nucleic Acids Res.* **28**, 235–242 (2000).
30. Zhang, Y. Progress and challenges in protein structure prediction. *Curr. Opin. Struct. Biol.* **18**, 342–348 (2008).
31. Marti-Renom, M.A. *et al.* Comparative protein structure modeling of genes and genomes. *Annu. Rev. Biophys. Biomol. Struct.* **29**, 291–325 (2000).
32. Altschul, S.F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–3402 (1997).

33. Rebecca Nelson et al: Structure of the cross-b spine of amyloid-like fibrils, NATURE|Vol 435|9 June 2005.

34. Buxbaum, J. D. et al. Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. J. Biol. Chem. 273, 27765–27767 (1998).